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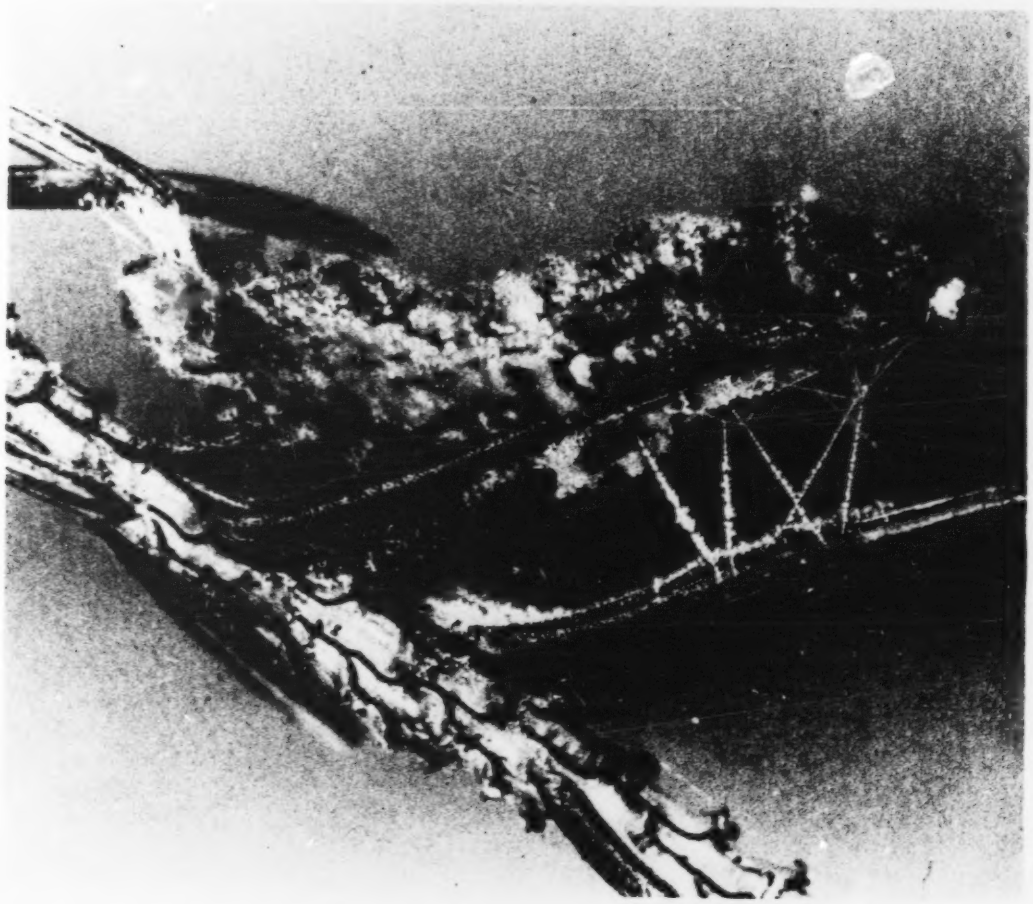

DEVELOPMENT OF ENVIRONMENTALLY ACCEPTABLE METHODS FOR CONTROLLING INSECT PESTS OF FORESTS

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A. Retnakaran, G.G. Grant, T.J. Ennis, P.G. Fast, B.M. Arif, D. Tyrrell and G.G. Wilson

Forest Pest Management Institute

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INTRODUCTION

The forests of Canada extend as a green belt across the nation occupying 3.14 million km^2 of land. This area is slightly larger than the total area of either India or Argentina. From such a vast resource, in 1979 alone, the forest industry earned 3.05 billion dollars and employed 1 out of every 10 Canadians (Reed *et al.* 1980a; 1980b). In order to obtain a sustained yield from this renewable resource, the forests have to be meticulously managed and regenerated.

Tree harvests are regulated by allowing an annual cut less than the annual growth. The harvested trees are replaced by reforestation programs carried out by the provinces.

Fires and pests take their toll on the forests and in many instances the two problems are interrelated. For example, a spruce budworm-killed stand of dead trees can easily ignite during a lightning storm and burn over a large area. The year 1967, when fires destroyed over 885,000 ha, resulting in a loss of more than 18 million dollars was a particularly bad one (Anon. 1970). The fires are detected by early warning systems and are controlled by various types of fire fighting units including aircraft.

The extent of damage to forests caused by insect pests is phenomenal. It has been estimated that the spruce budworm alone has been responsible for the loss of 560 million cords since the beginning of this century (Blais 1973). Large-scale control of forest pests, especially the spruce budworm, was initiated in 1940 using DDT. Even with much reduced dosages, during the fifties, repeated applications of DDT in New Brunswick resulted in a decline in the salmon population. The persistent nature of this insecticide was soon realized and it was replaced with compounds such as fenitrothion and matacil that break down rapidly in the environment (Prebble 1976).

At present, these broad spectrum insecticides are needed to protect the forests since there are no economically viable alternatives that can be used on a large scale. The search for alternatives to present-day chemical insecticides must be aimed at methods which avoid their detrimental side effects (Hudak and Raske 1980). This means the new approaches must be environmentally safe and non-toxic to non-target organisms such as birds, fish and beneficial insects (the predators, parasites and pollinators). Of course, they must also be safe to humans. In addition, it is now clear that no single method of control will be useful for every pest species--each insect will require an approach that is compatible with its habits and peculiarities. Thus, there is a need for a variety of control alternatives that will match the array of damaging insects that we have to contend with in our forests.

At the Forest Pest Management Institute in Sault Ste. Marie, in addition to improving the specificity of chemical pesticides, a team of scientists are systematically searching for alternative methods of control that are "non-destructive" to the environment. The goal of this research effort is to progressively reduce our dependence on broad spectrum insecticides. In this report we present a brief account of the various alternative methods and their potential for control.

PHYSIOLOGICAL METHODS

Some of the new approaches to pest control exploit the physiological and genetic systems characteristic of the insects themselves which ensures that these methods will be highly target specific. The three alternatives considered here which fall into this category are the use of insect hormones and growth regulators, insect sex pheromones, and genetic control. These techniques disrupt the developmental or reproductive processes of the target insect so that either it does not grow and survive, or it cannot reproduce. The use of insect hormones, growth regulators, and sex pheromones require the application of chemicals into the environ-

ment but they are characteristically non-toxic, labile and specific for insects. Genetic control makes use of the insects themselves as the destructive agent so that no chemicals are introduced into the environment.

Insect Hormones and Growth Regulators

Attempts to narrow the spectrum of activity of control agents and make them at least insect specific, if not pest specific, have led scientists to search for such alternatives in different directions. The physiologists have looked for materials that block critical biochemical pathways that are unique to insects. At present two such systems have been identified that can be disrupted by mimics. The first is juvenile hormone which, in concert with other endocrine secretions, controls growth and development. The second is a class of compounds called benzoyl ureas that block cuticle synthesis during growth and development in insects. Both types of compounds have been grouped as Insect Growth Regulators.

Juvenile Hormone Analogs (JHA): There are 3 endocrine glands responsible for orchestrating the sequence of events during growth and development in insects. The neurosecretory cells in the brain secrete the prothoracotropic hormone, a peptide which is released into the hemolymph. This hormone stimulates the prothoracic gland, a diffuse organ situated dorsally in the thoracic region, to secrete the steroid moulting hormone or ecdysone. A burst of ecdysone secretion triggers the moulting process leading the insect to moult from one instar to another. The outcome of the moult is dependent on the titer of a terpenoid hormone, juvenile hormone, secreted by a pair of minute glands called copora alata attached posteroventrally to the brain. If the level of juvenile hormone is high then the moult results in another larval stage. If the level is low then the insect pupates. If on the other hand it is absent, it moults into an adult (Gilbert 1976; Hammock and Quistad 1982).

Carroll Williams (1967) suggested that juvenile hormones could be used for insect control and he referred to them as "third generation pesticides". The real impetus to this suggestion came when a synthetic analog which was several fold more active than the authentic hormone was developed by Bowers (1969). Since then over 500 analogs have been synthesized.

If a juvenile hormone analog (JHA) is applied to the last larval instar where the titer of juvenile hormone (JH) is low, the metamorphic process is drastically altered. The outcome of the moult is a larval-pupal mosaic which seldom survives for more than a few days (Fig. 1). It will be most effective on insects in which the adult stage is the damaging form such as mosquitoes. In the case of forest insects no foliage protection can be realized in the year of application but if immigration of moths into a treated area is minimal it is possible to protect the forest in the subsequent year.

Several JHAs were tested against the spruce budworm with varying degrees of success (Retnakaran *et al.* 1978). This insect is apparently quite refractory to these materials and requires a relatively high, uneconomic level of material for control. The Eastern hemlock looper, on the other hand, is very sensitive to this material and can be well controlled with low dosages (Retnakaran *et al.* 1973). Again, foliage protection can be realized only in the year succeeding the spray, barring immigration of moths into the treated zone.

Yet another function of JH is in the determination of adult diapause in the white pine weevil. The adult insects emerge from the leaders during early fall, drop to the ground and overwinter in the duff. This behavior is in response to an absence of JH in the weevil. If however, these insects are treated with JHA, they become sexually mature, lay eggs and lose their ability to overwinter (Retnakaran 1974). In order to use this method in the field, the exact conditions under which the beetles go

underground to overwinter and the synchrony with which it occurs should be understood.

Anti-Juvenile hormones (Anti JH): Chemicals that negate the activity of juvenile hormone have been called anti-juvenile hormones (anti JH). Some dichromenes isolated from a garden bedding plant had anti JH activity on milkweed bugs (Bowers 1976). When early instars were treated with the material they moulted into miniature sterile adults instead of moulting into the next larval stage. Unfortunately this particular material (precocene) works only on certain bugs. More recently, it has been shown that fluoromevalonate acts as a potent anti JH, inhibiting JH synthesis (Quistad **et al.** 1981).

Although there is no anti JH being developed at present on a commercial scale, the prognosis for such compounds appears excellent. Treating young larvae and making them moult into sterile adults would virtually eliminate the destructive phase in most agricultural and forest pests.

Moult Inhibitors (MI): In 1973, it was found that some benzoyl ureas being developed for herbicide activity, when tested on insects, disrupted the normal moulting process. This serendipitous discovery led to the development of Dimilin®, the harbinger of all moult inhibitors (MI).

All insects have an exoskeleton that is reinforced with a polymer of N-acetyl glucosamine called chitin. Benzoyl ureas inhibit the polymerisation step during the biosynthetic process. The inability to synthesize chitin by larvae that have ingested this compound results in the integument becoming so fragile that fractures occur in weakened areas at the time of moult leading to the mortality of the insect (Maas **et al.** 1980, Retnakaran 1981).

In our laboratory we tested a number of moult inhibitors (MIs) against the spruce budworm, forest tent caterpillar and the oak leaf shredder (Retnakaran 1981, Retnakaran and Tomkins 1982, Retnakaran **et al.** 1979). Dimilin® was found to be very effective against the forest tent caterpillar and the oak leaf shredder. The spruce budworm proved to be refractory to most MIs. Recently we tested a compound, identified only by number as UC-62644, against the spruce budworm and found it to be very effective. Treated larvae become so fragile that it is difficult to pick them up with a camel hair brush without breaking the integument (Fig. 2). Field trials of this material demonstrated excellent control potential.

Although benzoyl ureas are relatively non-toxic to birds, mammals and most adult insect parasites and predators, many of these compounds have an adverse effect on certain juvenile crustacea and therefore caution should be exercised in treating areas inhabited by sensitive crustaceans.

Sex Pheromones

Most of the vitally important behavioral activities of insects are regulated by a variety of odorous chemical messengers, collectively called semiochemicals (from the Greek **semeion** meaning signal). Among the most important of these are the sex pheromones, the chemical signals transmitted from one member of a species to another to initiate and control mating behavior. In moths, the sex pheromone is released into the atmosphere from a specialized gland near the genitalia of the female (Fig. 3). The gland contains only a small quantity of pheromone at any one time and as this evaporates into the air and is carried downwind it is continuously diluted. Yet remarkably, males as distant as 100 m or more can detect and respond to the few hundreds of molecules of pheromone that reach them. This is possible because of the exceptionally well developed male antennae (Fig. 4) which are covered with thousands of olfactory cells specifically tuned to detect the pheromone. Once exposed, the male responds by flying

upwind, keeping inside the plume of odorous molecules until he reaches the source. Here, still under the influence of the pheromone, he courts and mates with the female.

Since sex pheromones have such powerful long-range effects, requiring only a minute quantity of material, it was quickly realized that these compounds offered a safe method of controlling insect populations by manipulating their behavior. The practical application of sex pheromones for insect control can be divided into two categories: population survey or monitoring, and population suppression (Silverstein 1981). In survey use, traps with sticky surfaces are baited with the pheromone of the target species and distributed wherever the pest may occur such as in forests, plantations, seed orchards, tree nurseries, or parks. The traps are checked periodically and the catch identified and counted (Fig. 5). The trap catch can be used to indicate the presence or absence of a particular pest and to determine its geographical distribution. Currently, this is the approach being used to follow the spread of the gypsy moth infestation across Canada. When the insect is discovered where it could cause damage, control action can be undertaken. Alternatively, the trap catch can be calibrated, by comparing catches from year to year, with the population density of the immature feeding stage (larva) and with the degree of associated defoliation. This type of information is most useful for predicting outbreaks and for planning corrective operations before the population gets out of control. It is far less expensive to apply preventative measures than it is to try to control a population after it has reached severely damaging levels. Monitoring insect populations with sex pheromone baited traps has provided an inexpensive and easy-to-use tool which can replace other more time consuming and costly survey techniques. It is most advantageous at very low population levels which cannot be detected by other means.

The second and more exciting use of pheromone is for the direct suppression of an insect population by preventing mating (Mitchell 1981; Silverstein 1981). There are two ways of achieving this. In areas which are easily accessible and limited in size, such as tree plantations and seed orchards, it is feasible to deploy throughout the treatment area enough traps baited with sex pheromne to remove (trap out) sufficient males so that very few of the local females are mated. If mated females from outside the treated area do not fly in, the subsequent population will be reduced to well below damaging levels. To investigate the potential of this approach in a forestry situation, we are undertaking a trapping out experiment against a species of pine shoot moth which infests plantations of young red pines and which cannot be easily controlled with conventional insecticides or other means. Since the pheromone is confined to the traps and is used at very low dosages, this technique is one of the safest approaches to insect control for the environment, wildlife and man.

Unfortunately trapping out is not feasible over large areas. The alternative method of preventing insect mating is known as the "mating disruption" or "male confusion" technique in which pheromone is dispensed into the atmosphere elevating the levels well above that produced by the females themselves. Since the male cannot distinguish between the artificially introduced pheromone and the natural pheromone of the females, the chemical communication system of the species is disabled and the male cannot locate the female. Pheromone can be dispensed into the environment by encapsulating it in gelatin-based micro beads, in very small hollow fibres, or in confetti-like laminated chips (Fig. 6), all of which can be aerially applied. Disruption of mating by atmospheric permeation with pheromone is appealing because to date pheromones have been shown to be non-toxic to mammals, biodegradable, highly target specific and require much lower dosages than conventional chemical insecticides (Mitchell 1981).

An important first step in the development of pheromones for insect control is to chemically identify their structure. Through collabora-

tion with other laboratories in Canada and the U.S.A., FPMI has had a highly successful project devoted to this goal. In the early 1970's, the sex pheromone of the spruce budworm was identified (Sanders and Weatherston 1976). This led to its use across Canada as a supplement to other more costly survey techniques. Additional forest insects for which sex attractants have been found include the forest tent caterpillar moth, the oak leaf shredder, the maple leaf roller, the eastern pine shoot moth, the pine tussock moth and the western spruce budworm, among others. In some cases, such as the oak leaf shredder, which is a serious pest of oak in parks and other areas of southern Ontario, the pheromone is now being incorporated into survey traps so that population build-ups can be monitored and outbreaks predicted. Similar work is being carried out with the spruce seedworm, an insect that infests cones and destroys the seeds. Since the forestry industry is currently engaged in a significant effort to regenerate Canada's forests, it is essential that we protect the seeds required to provide the nursery stock for their regeneration. In other cases, the identified pheromones are being used to explore the potential for mating disruption to control such insects as the tussock moths and pine shoot moths. For example, we have found that small quantities of (Z)-6-henelcosen-11-one, a principal component of the sex pheromone of six tussock moth species can disrupt the chemical communication system of several eastern tussock species (Grant 1978). Similarly, the recently discovered sex attractant of the eastern pine shoot moth caused a 50% reduction in trap catch in small plots treated with only 0.04 g AI/ha compared to control plots, indicating its suitability for mating disruption in this species.

The potential usefulness of insect sex pheromone has been established, but a great deal of fundamental research is still required to bring pheromones into the arsenal of viable control alternatives. For example, we frequently find that mating disruption fails at high population levels but we do not know why. The problem here is that we lack sufficient information about the behavioral response of insects to pheromones and about the mechanism by which the disruption process operates. Nonetheless, sex pheromones are close to satisfying the promise of a safe and environmentally acceptable alternative to present day chemical insecticides for some insects. They may have an even wider application and usefulness if they are combined with one or more of the other techniques discussed in this article in an integrated pest management system.

Genetic Control

Those insects which compete with man for some aspect of the forest resource do so because they are successful in an evolutionary sense. They are well adapted to the forest environment, usually because of high reproductive potential --each mated pair gives rise to large numbers of eggs and larvae--and because they are genetically resilient--they can maintain their numbers when environmental conditions are adverse and increase when conditions become favorable. Many of the methods of control discussed in this article attempt to regulate insect numbers by enhancing natural mortality, by interfering with normal growth and development or by disrupting mating. The genetic control approach is aimed directly at the insect's genetic system, either by interfering with reproduction and the production of viable eggs or by genetically weakening some stage of development to make it more susceptible to environmentally induced mortality (Knipling, 1979). This latter approach, modification of the genetic systems, requires a level of genetic knowledge and sophistication that is not available for any forest insect pest, so emphasis here will be on interference with reproduction.

In concept, the use of genetics to interfere with reproduction of natural populations is simple. It involves the mass rearing of sterile or sterilized insects to the adult stage and their release into natural populations in numbers sufficiently high that they will successfully compete with the normal wild insects

in mating. This should lead to the production of sterile or non-viable eggs and thus reduce the reproductive rate (Davidson 1974). The approach has been termed the Sterile Insect Technique (SIT). Its greatest advantage lies in the fact that it is absolutely species-specific and environmentally non-contaminating. However, as with many apparently simple approaches, its development as a useable management tool has in many cases met with setbacks and difficulties.

Complete sterility can be induced by treatment of the adult stage with cobalt-60 or x-irradiation (Smith and Von Borstel 1972) or by use of chemosterilants (LaBreque and Smith 1968). As irradiation leaves no residue and can be safely and precisely controlled, it is generally preferable to the use of chemosterilants, many of which are toxic to mammals and potentially carcinogenic. Dosages used are adapted to the specific insect to be treated, as there is a great deal of variation in susceptibility. At excessive dosages, accompanying somatic damage reduces the treated insects competitiveness. Though originally limited to use of males, SIT can also include release of females for some insects, as long as the released females are sterile or nearly so.

The first major success of SIT was with the screw worm (Davidson 1974), a parasite of warm blooded mammals such as cattle from the southern parts of the United States to South America. In a program initiated in 1957, weekly releases of up to 50 million radiation-sterilized males into infested areas of Florida led to complete eradication of the pest after seventeen months. A similar program near the Texas-Mexico border involved the release of 150 million flies per week. Initiated in 1962, this program still continues, and the pest has been drastically suppressed, if not eradicated, in the southwestern United States and Northern Mexico. Economic success has been equally impressive with a calculated benefit/cost ratio of 100.

Efforts with other non-forest insects have been variably successful (Davidson 1974; LaChance 1979). A number of fruit flies have been suppressed, including the melon fly in Japan and the Mediterranean fruit fly in Mexico. Among vectors of human disease, malarial mosquitoes and the tsetse fly are currently the target of developing SIT programs. In agriculture, the cotton boll weevil and the codling moth have both been the objects of SIT programs. Though eradication or suppression of localized populations have been achieved for all of these insect pests, the widespread success achieved with the screw worm has not yet been repeated. This can be attributed to a number of factors, but the most important is the need to develop a technique that is specifically adapted to the particular target insect and takes into account not only its genetic system but also all aspects of its behavior in the wild.

Use of SIT against forest insect pests has received little attention, due in part to general lack of basic genetic information for most species. Additionally, outbreaks of economically important forest insect pests tend to cover large geographic areas and involve tremendous numbers of insects, posing formidable problems of effective application. Preliminary studies at the Forest Pest Management Institute have established effective methods of sterilization of the spruce budworm (Retnakaran 1970, 1971) and provided laboratory evidence that SIT can be used to reduce fertility of caged populations (Ennis 1979a, b). However, there is no present plan to extend these tests to the field.

Genetic control remains a technique with great theoretical potential, but has proven a success in only a limited number of cases. Because of its advantages, efforts will continue to develop SIT for specific insects. The target insect will have to be chosen on the basis of specific criteria for suitability (Knipling 1979). These will include amount of knowledge of behavior and dynamics of the insect, its distribution and numbers at economically important levels, and the relative ease with which it can be controlled by alternative techniques.

BIOLOGICAL CONTROL

All insect populations are subject to what we might call the "natural enemy complex", the members of which all contribute to the overall mortality suffered by the population and, in normal circumstances generally maintain insect populations below levels of economic consequence. The effect of releasing insects from this controlling enemy complex can be seen in the massive damage done to our forests by such insects as the gypsy moth and the European spruce sawfly, which were accidentally introduced into North America where most of their natural enemies did not exist. Members of this complex include the disease causing microorganisms, bacteria, fungi, viruses and microsporida, together with parasites which are usually other insects, and predators, which include insects, birds, some reptiles and small mammals. Because these agents are already present in nature and in most cases do an efficient job of keeping their insect hosts in check we are actively seeking ways in which we can use them to our own advantage, especially in situations where man through his activities has tipped the balance in favour of the insect by, for instance, creating large tracts of a single species of tree, or where the insect's function in nature conflicts with man's designs, as in the case of the spruce budworm, whose natural role in the forest cycle conflicts with our desire to utilize those same trees for pulp and timber.

While some of these natural control agents are relatively non-specific with regard to insect host, many are narrowly, or even species, specific. Because they are already part of the environment, and because they often do possess a high degree of specificity, a considerable research effort has been expended to determine how we can manipulate these organisms to make use of or enhance their effectiveness in insect population control without danger to man or to the environment or without simultaneously removing other members of the parasite/predator complex, as happens frequently with broad spectrum chemical insecticides.

Bacteria

The bacterium **Bacillus thuringiensis** is the most widely used and highly developed of the microbial insecticides, enjoying a large market in agricultural applications. This has occurred because of the ease of production, safety, and the high and selective toxicity to susceptible insects, primarily the larvae of moths (Lepidoptera), mosquitoes and blackflies. Many of the susceptible lepidopterans are very important agricultural pests, and the blackflies and mosquitoes are major vectors of many tropical and subtropical diseases.

Commercial preparations of this bacterium consist of spores and protein crystals formed by the cells during the process of sporulation, along with inert ingredients that function to preserve and facilitate application of the spores and crystals to crops. The protein crystal (Fig. 7) is the primary active ingredient without which the preparations are not toxic. The preparation must be ingested by the target insect to be active; it has no contact toxicity. It is dissolved in the gut of the target insect by a combination of high pH (greater than 8.5) and proteolytic enzymes. The dissolved toxin acts on the cells lining the gut and very rapidly kills those that are contacted. This causes the insect to cease feeding within 15 minutes after consuming a very small dose. Once feeding ceases the insect will, in most cases, die without causing further damage. The protection of the crop is therefore very rapid even though the insect may not die for several days after it has ingested a dose.

Insects in which the gut pH is more neutral are not susceptible because the crystal, properly a protoxin, cannot be dissolved under these conditions. When such insects are challenged by toxin produced by dissolving crystals in the gut of susceptible species they also are found to be susceptible. In animals in which the first section of the gut is acid and pepsin is active, such as mammals, the crystal is degraded to an inactive form. Even if that were not the case,

vertebrate cells do not appear to be susceptible to the toxin even after it has been activated by insect enzymes. Thus the host range of this bacterium is restricted to some larvae of Lepidoptera (moths and butterflies), one species of white grub, and some larvae of mosquitoes, blackflies, and midges. A particular isolate of **Bacillus thuringiensis** has recently been found that is very highly toxic to mosquitoes, blackflies and midges with no known toxicity to other organisms, even the Lepidoptera.

This very restricted host range is also the basis for the safety of this organism. With its lack of toxicity to fish, birds, mammals and most other vertebrates and invertebrates, the Environmental Protection Agency of the United States Government has allowed this material to be used on agricultural crops right up until harvest. It can be used over vast acreages of forest or repeatedly over restricted acreages for intensive insect control with no adverse effects either on natural enemies of the pest or on the environment. Because natural enemies are not affected, the need for additonal applications of insecticide are reduced, contrary to what is observed with chemical insecticides where the predaceous and parasitic insects are killed along with the pest and the need for insecticidal applications is increased.

This bacterial insecticide is relatively simple to produce. It is fermented in large tanks, in the same manner as antibiotic producing organisms, using waste products of the food or agriculture industries as raw materials. At the end of the vegetative stage of growth the cells sporulate and produce the crystal. The mixture of spores and crystals and insoluble fermentation residue is harvested either by centrifugation or by spray drying, and then formulated to facilitate application and deposition on the target foliage. There are 2 major manufacturers in the U.S., as well as several smaller producers, who together produced an estimated 1.5 million pounds of **Bacillus thuringiensis** in 1980. The main markets are for use on vegetable crops, cotton, tobacco, soybeans, stored grains and areas of forestry. As more potent products come on the market as a result of biotechnology and genetic engineering, bringing down the cost of production, the market can be expected to expand. One can anticipate that forest spraying of this material will increase as application technology improves so that this organism can be applied in lower volumes and more effective deposition can be obtained.

A detailed account of the use of bacteria for insect control has recently been summarized in a book edited by Burges (1981).

Baculoviruses

The need for a viable alternative to chemical insecticides in the fight against forest and agricultural insect pests has sparked a great deal of interest in insect viruses generally and more specifically in the baculovirus group. This group, nuclear polyhedrosis (NPVs, subgroup A) and granulosis viruses (GVs, subgroup B) (Fenner, 1976) are rod-shaped particles 250-400 nm long with a dense DNA containing nucleocapsid and a multilaminar membrane. The viruses are embedded in a crystalline protein inclusion body called polyhedra in the case of NPVs and capsule in the case of GVs. The polyhedra contain more than one virus particle while the capsule contains only one and very rarely two particles. There are also two forms of NPVs that become occluded in polyhedra. One form contains one nucleocapsid per membrane (SNPV) and the other contains more than one nucleocapsid per membrane (MNPV) (Figure 8). The infectious unit is the virus particle that is embedded in the inclusion body (Arif and Brown, 1975). Isolate nucleocapsids are not infectious which indicates that the sites of viral attachment to cell reside on the surface of the virus (Arif and Brown 1975).

Physical Properties of the Virus: It is essential that we have a comprehensive understanding of the biochemical nature and biophysical proper-

ties of these viruses before their wide spread use as a biological insecticide. These studies not only will yield information and insight on the nature of these viruses but will also serve to identify and classify them.

1. Viral Proteins: Studies have shown that the inclusion body protein is a crystalline matrix of a single subunit (Summers 1975). The biochemical nature of this protein has been well studied (Summers and Smith 1978; Harrap **et al.** 1977). This protein is believed to protect the virus particles (virions) from damaging environmental factors such as dehydration and sunlight. An alkaline proteolytic activity was found associated with this protein (Eppostein and Thoma 1975). The function of this enzymatic activity and its role in the infectious cycle has so far been a matter of speculation and is not clearly understood.

The virions, as expected, revealed a very complex structure when analyzed by SDS gel electrophoresis. Summers and Smith (1978) demonstrated the presence of 15-25 viral structural polypeptides of varying molecular weight. Many NPVs have a unique polypeptide composition and in some cases the viruses are readily distinguishable by their protein pattern. For example, MNPVs from several larvae have major differences in their polypeptides that can be used for identifying the viruses from the different host insect species. Nucleocapsids isolated and purified from the virions showed the presence of 1-5 polypeptides.

2. Viral Genome: The DNA of baculoviruses is found in the nucleocapsid and is a double stranded closed circular molecule. The molecular weight of most NPV DNAs is in the order of 80 x 10^6 (Harrap and Payne, 1979). Recent advances in the study of baculovirus DNA have produced physical and genetic maps (Smith and Summers, 1979; Miller and Dowes, 1979). These studies will not only be useful in the identification of different virus isolates but will aid significantly in understanding the function of these viruses in the infected host.

3. Replicative Cycle: In nature the viral inclusion bodies are ingested by larvae where they dissolve in the alkaline environment of the gut releasing the occluded virions. The virions will initially infect the gut epithelial cells and then invade the other tissues of the larva. The infected cell produces two forms of viruses. One becomes occluded in polyhedra and is destined for dissemination and infection of other larvae whereas the other is non-occluded, the so called non-occluded virus or NOV (Volkman **et al.** 1976). It is through this NOV that infection is spread through the various tissues of the larva. NOV is also the form of virus used to infect insect tissue culture cells (Granados 1976). In the infection cycle NOV is absorbed on the surface of the cell, enters and is then transported to the nucleus where the DNA of the virus loses its coat. Synthesis of viral specific polypeptides in the host cell appear 3 hours post infection. This synthesis appears in a specific sequence and by 32 hours post infection 35 different viral specific proteins can be detected. The last to be synthesized appears to be the inclusion body protein (Carstens **et al.** 1979). By this time there is ample supply of mature virus which becomes occluded in polyhedra. NOV in the meantime is released to infect other cells.

4. Specificity and Safety: NPVs and GPVs are highly specific insect viruses which infect only members of the host family. This specificity makes them very attractive alternatives to broad spectrum chemical insecticides. Indeed, tests conducted to assess the effect of NPVs on vertebrates have all been negative and as a result many of these NPVs were registered in the USA as biological insecticides (e.g., NPVs of the gypsy moth and of the Douglas fir tussock moth). In Canada similar tests were conducted on two NPVs, one of the spruce budworm and the other of the red-headed pine sawfly. These tests have unequivocally shown the safety of these two viruses to mammals, birds and fish. As a result a petition was submitted to register the NPV of the red-headed pine sawfly, **Neodiprion lecontei,**

as a biological control agent under the Pest Control Act of Canada. This NPV of the sawfly is the most efficacious virus known. It is very effective in controlling sawfly infestations (Kaupp **et al.** 1978) and can be produced at relatively low cost. Indeed this virus is so successful that continual application of this virus could result in the eradication of the sawfly in Ontario.

The NPV of the spruce budworm is not as efficacious as that of the sawfly. One way to increase its potency is to improve its virulence. This may be done by producing mutants of the virus which have alterations in their gene structure that renders some of them more virulent. Before embarking on such experiments it is essential to understand the basic nature and function of these viruses. Another alternative is to genetically manipulate the DNA to produce more effective virus. This latter approach basically involves excising the DNA with restriction endonuclease enzymes and introducing new DNA segments from another more virulent, and maybe closely related virus, and then ligating the new DNA piece in order to become a continuous part of the genome. Since baculoviruses are so highly insect specific, are morphologically distinct from all vertebrate viruses, and their safety is well established, they are excellent models for such an approach.

Fungi

Entomogenous fungi are widely distributed in nature, and are probably the best known of the disease-causing microorganisms to the non-specialist, because infected insects often are covered with fungal mycelium and present a rather unusual appearance as the fungal fruiting bodies proliferate on the outside of the insect (Fig. 9).

There are a large number of species of fungus which attack and kill insects, and their effect ranges from the occasional infected specimen to the spectacular epizootics which sometimes occur in high insect populations and in which dead insects may literally be collected by the bucketful. A common example of the latter frequently occurs at the end of the outbreak phase of the forest tent caterpillar, and is caused by the fungus **Erynia crustosa.** Similar occurrences have been observed in the eastern hemlock looper and other forest insects.

Fungi differ from other microorganisms in that they are not dependent on insect feeding to gain entry to the insect body, although some species can penetrate the gut wall. The reason for the success of entomogenous fungi is that they have developed an enzymic cocktail which enables them to penetrate the body of the insect directly through the integument. They are thus not limited to attacking the insect at its feeding site, but can potentially attack and make infection throughout the entire activity cycle of an insect. In addition, fungi are the only major pathogens of sucking insects, such as aphids, whose feeding habits enable them to bypass surface contaminations of virus and bacteria (Dulmage **et al.** 1981).

It is obvious then that fungi possess considerable potential as biological control agents for insect pests (Fetton 1978). Indeed, the first attempts to use fungi for this purpose date back to the last century, in Russia (Cameron 1971), but until recently the results have been somewhat equivocal at best, and frequently disappointing. A major reason for the discrepancy between expectation and result has been a lack of systematic study of the relationships between entomogenous fungi, their insect host and the other biotic and abiotic factors which influence their interaction. As additional information becomes available, a more rational approach to application strategies becomes possible. The appearance of several commercial fungal preparations on the market in the last few years suggest that these problems are being successfully overcome.

In considering the application of fungi as biological control agents, two strategies are

possible: auto dissemination and colonization, or as a mycoinsecticide (Yendol and Roberts, 1970). In the former approach, advantage is taken of the fact that fungi are living organisms that can grow and multiply in our pest population. Infection foci are established, and the disease is allowed to spread unassisted throughout the population. This method is economical in terms of material and labor and is especially useful in cases where, for instance, the fungus cannot be grown in artificial culture and dissemination must be via infected insects. If live infected insects are used, they have the further advantage that they will carry the fungal inoculum directly to the habitat of the target species. This approach will have maximum utility in cases where introduced insects have established unhindered by their natural enemy complex, or in cases where, because of spatial separation or other factors, a fungus does not infect the host population early enough to prevent economic damage from occurring, despite the fact that the earlier stages of the insect are susceptible to fungal attack. It is an attractive strategy for attacking forest insect pests where it may be possible to establish an infection over large areas from relatively few point sources.

The second approach to the use of fungal pathogens is analagous to the use of conventional insecticides. Spore preparations of suitable entomopathogens are formulated and applied to pest populations by spraying in the conventional manner, usually in sufficient quantities that the ability of the fungus to establish and spread in the population is secondary to the requirement for maximum infection to be obtained at the time of spraying. This method is the easiest to manipulate and commercialize, but is restricted to those species of fungus which can readily be grown in quantity on artificial media. The commercial preparations mentioned above have been developed with this approach in mind for use primarily in agricultural situations, but the fact that they are now available commercially points out that we are beginning to realize some of the potential of entomogenous fungi in biological control; and, in time we can expect fungi to contribute to a much greater extent in the biological control of forest insect pests.

Microsporidia

Numerous species of protozoan parasites are associated with insects; of these the microsporidia are the most common. Most insect species are host to at least one microsporidium and certain of these undoubtedly play a significant role in the natural suppression of some insect populations. Microsporidia generally produce chronic rather than acute disease and therefore, as control agents, do not create as dramatic results as do some of the viruses, bacteria and fungi. In fact natural epizootics caused by protozoa have seldom been documented. Although microsporidian infections are usually chronic, some may be severe and fatal and probably are more important than is generally realized. Laboratory studies have shown that without being spectacular, microsporidian infection can have profound effect on insect populations. This is usually manifested by one or more of several forms; reduced survival, irregular growth, loss of appetite, smaller and malformed adults, and reduced fecundity. Due to the chronic, debilitative nature of infection, most microsporidia offer little potential as short-term, quick-acting control agents, their use offering more promise as long-term control or as part of an integrated control program. Only recently have a few attempts been made to utilize microsporidia as microbial control agents (see recent reviews of Brooks, 1980; Henry, 1981 and Wilson, 1982).

Species of microsporidia commonly occur in Canadian forest pest insects. **Nosema fumiferanae** is unquestionably the most common parasite of the spruce budworm (Fig. 11). **Pleistophora schubergi** will also readily infect the spruce budworm, forest tent caterpillar and other forest pests. Both microsporidia adversely affect larval survival, pupal weights and reduce adult longevity and fecundity.

It has been observed that levels of infection by N. **fumiferanae** fluctuate in budworm populations, increasing with the age of the infestation. For example, examination of living spruce budworm, collected in the field over a six-year period, showed levels of infecton increased from 35.9% in 1973 to 69.0% in 1978. It is not yet clear what effect these levels of infection have on the insect population, but based on laboratory studies, it is probable that the microsporidium plays a role in regulating the population possibly by reducing its overall vigour.

Treatment of diet surface, on which 2nd instar spruce budworm are allowed to feed, with 2 x 10^5 N. **fumiferanae** spores will reduce oviposition in resulting adults by 25%. If the same dosage is fed to naturally infected insects oviposition is reduced even more and adult longevity is shortened by about 3 days. However, larval mortality is not significantly increased unless large dosages of spores are used. The microsporidium P. **schubergi** has a greater effect on mortality than N. **fumiferanae**. For example, in the case of spruce budworm a treatment of 2 x 10^8 P. **schubergi** spores applied to the surface of the host's diet resulted in 100% mortality when fed to 2nd and 60% to 5th instar larvae. With forest tent caterpillars 5 x 10^7 spores resulted in 100% mortality when fed to 2nd instar larvae. Spores of the microsporidium **Nosema disstriae** fed to 2nd instar tent caterpillar larvae adversely affect pupal weights, adult fecundity and longevity.

Preliminary field trials have been conducted against the spruce budworm using **N. fumiferanae** or **P. schubergi**. Individual white spruce and balsam fir trees were treated with microsporidian spores. Significant increases in the levels of infection occurred in the larvae after treatment. The results of these field tests indicated that N. **fumiferanae** can be successfully introduced into a population of spruce budworm and thereby increase the levels of the microsporidium in that population. Since N. **fumiferanae** is transmitted transovarially, most offspring of infected adults are also infected. There was no carryover of P. **schubergi** following the initial high infection (up to 95%) in the year of spraying. Based on laboratory results, this may have been due, in part, to mortality of infected insects; however, larval reduction studies of field populations were not carried out.

A combination of two microsporidian parasites, **N. disstriae** and **P. schubergi**, was tested against the forest tent caterpillar. High infection rates were obtained for P. **schubergi** but not for N. **disstriae**.

Although we have only begun the study of microsporidia as applied and natural control agents, significant progress has been made in this field. The major role of microsporidia will be played in an integrated program because of the chronic nature of the disease caused by this organism.

Parasites and Predators

In the forest environment, insects whose feeding or egg laying habits inflict damage upon trees are generally considered to be pests. Their economic importance is determined by their abundance and by the extent to which this habit affects the growth or survival of the tree. In the complex ecosystem that is a forest, however, these same pest insects are subject to the feeding and/or egg laying habits of a great variety of other vertebrate and invertebrate species. These are considered to be predators when they directly consume the insect as a food source, or parasites, when a considerable portion of their life cycle is spent within or directly attached to the host insect, using it as a food source.

Perhaps the most obvious predators in the forest are the vertebrates, particularly birds. Pest insects such as the spruce budworm can provide up to 40% of the food intake of some insectivorous birds (Hudak and Raske, 1980). At relatively low pest insect levels, bird predation can exert significant stabilizing influences on population levels. Small mammals such

as mice and shrews can also have significant effects upon insect numbers. In Newfoundland, one species of shrew introduced from the mainland has been successful in depressing populations of the larch sawfly through predation on coccoons (Reeks and Cameron, 1971). The role of reptiles and amphibians in forest insect control is uncertain. Though they may be locally significant when abundant, it would not appear their predation is of much significance.

Predation by other invertebrates clearly is important in maintenance of pest insect levels, if for no other reason that their sheer numbers and diversity. With some exceptions, spiders are opportunistic feeders and though relatively nonselective, are expected to have effects particularly at low host levels. Mites can be significant egg predators of some agricultural pests, and probably play a similar role in forests, though to what level is unclear. Under some conditions, ant predation can be very significant.

Parasitism by other insect species, particularly the Diptera and Hymenoptera, can exert a very effective regulating force upon forest insect populations. These agents have received much attention for use in control of forest insect pests in Canada, and some notable successes have been achieved (Reeks and Cameron 1971). The European spruce sawfly and the winter moth have been reduced from epidemic, economically important, to very low endemic levels by the action of released parasites, though in both cases, a virus also exerts significant control. Effective parasites have been found for a number of other pest insects, and though control success has varied greatly, it seems clear that parasitism can be a useful and effective method of insect control, particularly at relatively low population levels.

Nematodes are known to be parasites in other insect pests, but their effectiveness against forest insects is virtually unknown. Recent attempts to use a nematode against the spruce budworm have shown promise in laboratory tests, but much additional study is required to determine their potential in field situations.

It is a characteristic of predator and parasite populations that they follow changes in numbers of their host insects. This is particularly true of those with specific requirements for the host. This cyclical dependence on host density is also usually marked by a delay phase which can be attributed to the reproductive delay of the parasite/predator in reacting to increased numbers of hosts. For small cyclical changes, the parasites can re-establish control over host numbers, but in an outbreak situation, rate of increase of hosts soon out-paces parasites and predators to a point where they no longer exert a controlling effect. The development of parasites and predators as potential control agents are therefore generally not aimed at epidemic insect populations. Rather, the best strategy is to enhance their numbers at relatively low host densities, where even a small increase in parasitism/predation can retard the increase in numbers of the host (Knipling 1979). In theory, this will allow enough time for the complex of natural enemies to regain their controlling effect over the host pest insect.

Though parasites in particular have in several instances shown great promise as control agents, there remain many forest insects over which parasites and predators exert little control in their most numerous and destructive phase. In such cases, the spruce budworm for example, their potential seems to be in their effect on low level populations and those that are just beginning to increase. Given evidence that parasitism and predation play important roles in the dynamics of transition from endemic to epidemic levels, future research should be aimed at understanding how they exert their influence. This should lead to development of a systematic approach to using parasites and predators to control economically important forest insect pests.

CONTROLS OF THE FUTURE

Fifty years ago, Professor Dwight Isely developed a multifaceted control program for the

cotton boll weevil. The system consisted of (i) planting of early maturing cotton, (ii) spot treating of high density weevil areas, (iii) controlled burning of cotton stubble that serves as overwintering sites, (iv) monitoring populations continuously and treating with insecticides as and when indicated, and finally (v) conserving natural enemies of cotton pests. This was the beginning of Integrated Pest Management (IPM). Although the principles of IPM were firmly established, it was ignored for the most part from 1940 through the mid 1960s. During the 1970s, with the success of several IPM programs, the concept has been enthusiastically accepted (Newsom, 1980).

IPM takes into account the ecological interaction of the pest with the host and identifies the various ways in which the economic injury threshold can be held at an acceptable level. In the context of forestry it involves cultural practices, strain selection of trees, judicious application of a panoply of control measures, protection of beneficial species, and minimum adverse effect on the non-target species. Because of their ecological acceptability, many of the alternative strategies discussed earlier, can be used in such an integrated control program. Modest beginnings have been made in the area of IPM with regard to species such as the gypsy moth, Douglas fir tussock moth, and the southern pine beetle and it is more than likely that this trend will be extended to other forest insects in the years to come.

Apart from all these techniques, the announcement of experiments involving recombinant DNA and molecular cloning in 1973 heralds a new era in the devlopment of pest specific control methods. Innovative techniques have been developed that make it possible in principle to transfer new genetic information into microorganisms thus enabling the synthesis of desired compounds in bacteria at a relatively low cost. These techniques of genetic engineering are powerful tools and can be used for not only understanding the structure and function of genes but can also be used to develop organisms with properties of commercial importance.

In the context of biological control of forest insects it is theoretically possible to produce more virulent and/or more UV resistant strains for control and this has been alluded to in the section dealing with baculoviruses. The tailoring of microorganism for insect control from the ones available calls for changing their genetic information in a way that accentuates desirable properties or introduces entirely new ones. For rational genetic programming a clear understanding of the characteristics of the DNA of the organisms under study is a prerequisite. In the years to come it is conceivable that genetically engineered microorganisms will become available for insect control.

In summary, the forest manager currently has a number of options available for control of forest insect pests. With the exception of B.t., these options primarily include conventional chemical pesticides, for which intensive research has produced application doses and methods that achieve the desired control efficacy with as little adverse effect on the environment as possible. The approaches described above, when successfully developed, will increase the control options available to the manager. This will allow him to develop control strategies that can optimize the effectiveness of chemicals, microbials, pheromones, parasites and predators to protect the forest resources, at the same time minimizing the adverse effects of this very necessary activity on the environment.

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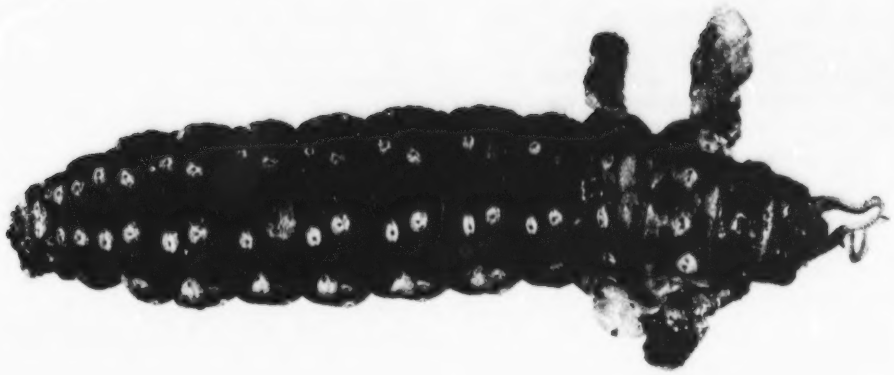

Figure 1. Effect of a juvenile hormone on the 6th instar larva of the spruce budworm. Everted wings and antennae give a bizarre appearance to this larva.

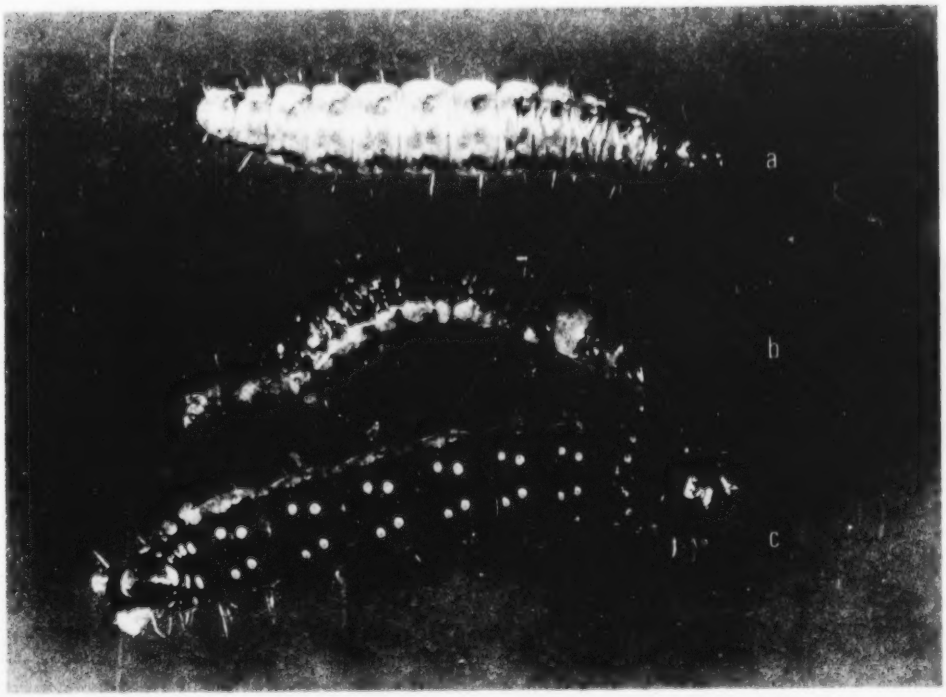

Figure 2. Effect of the moult inhibitor, UC-62644, on fifth instar larvae of the spruce budworm. a) Normal fifth instar b) Treated fifth instar showing weakened areas c) Normal sixth instar.

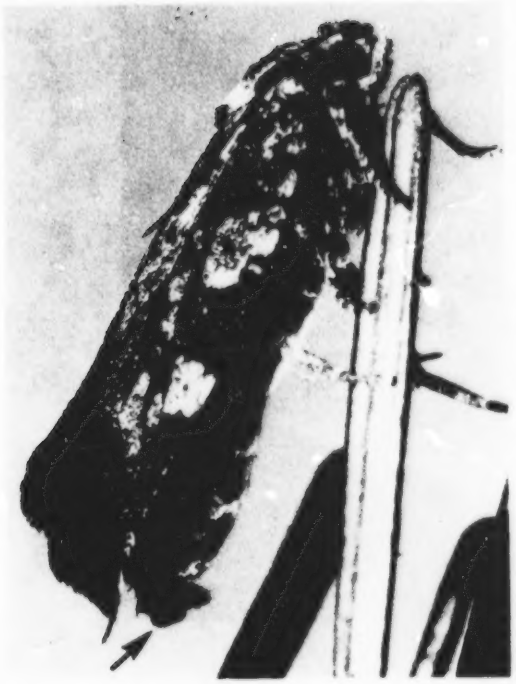

Figure 3. Sex pheromone gland of the female spruce budworm moth. The moth everts the gland and assumes a characteristic "calling" position.

Figure 4. Antennae of the male polyphemus moth. The receptors for the pheromone emitted by the female are situated in the plumose antennae.

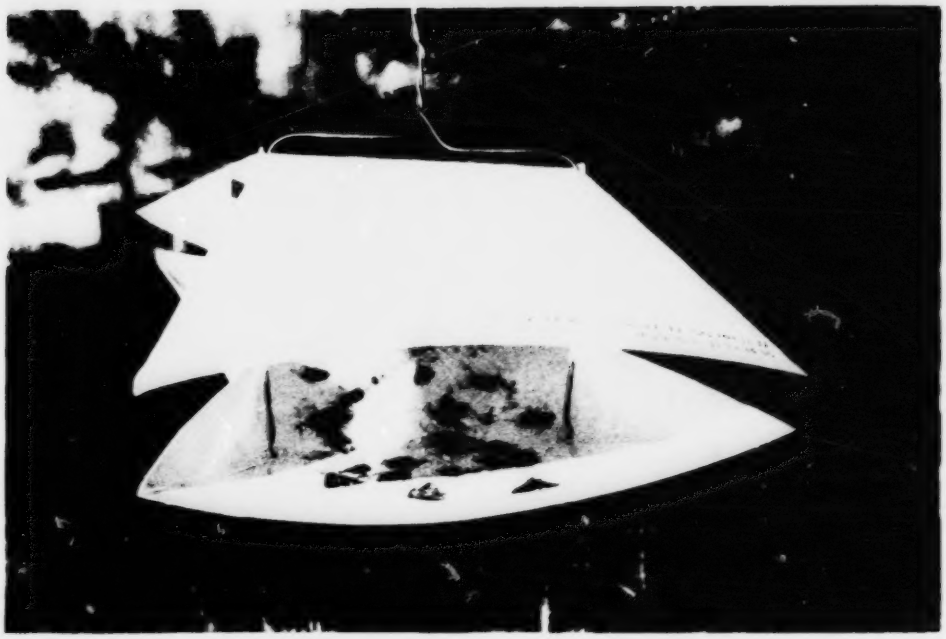

Figure 5. Catch of moths in a pheromne trap.

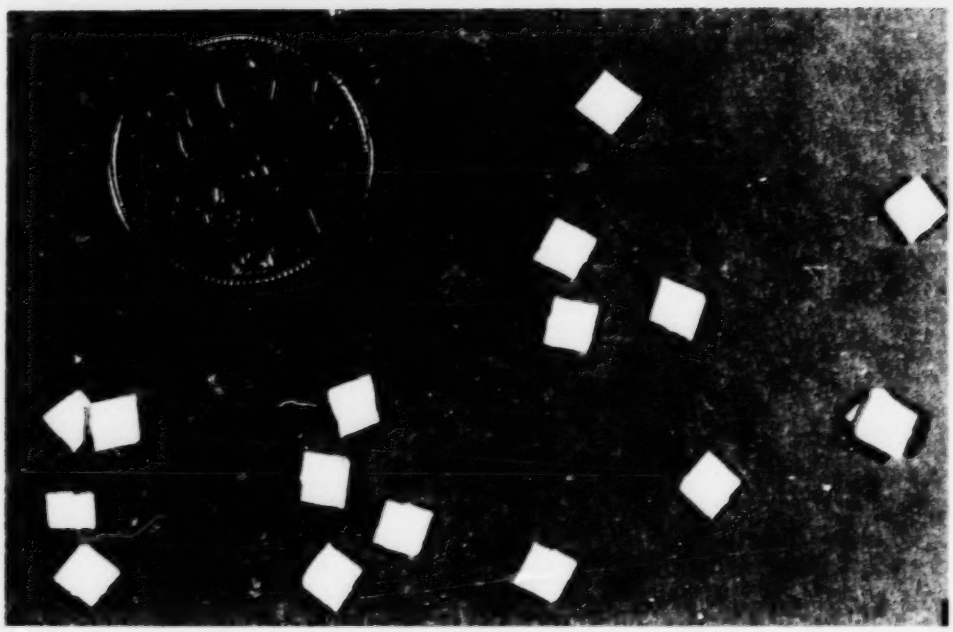

Figure 6. Hercon flakes containing the sex pheromone used for mating disruption.

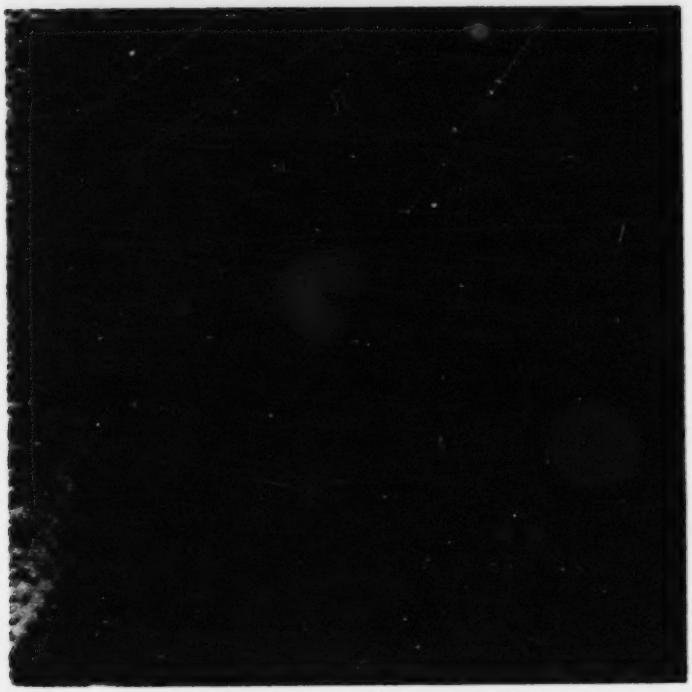

Figure 7. Electron micrograph of the crystal from **Bacillus thuringiensis.**

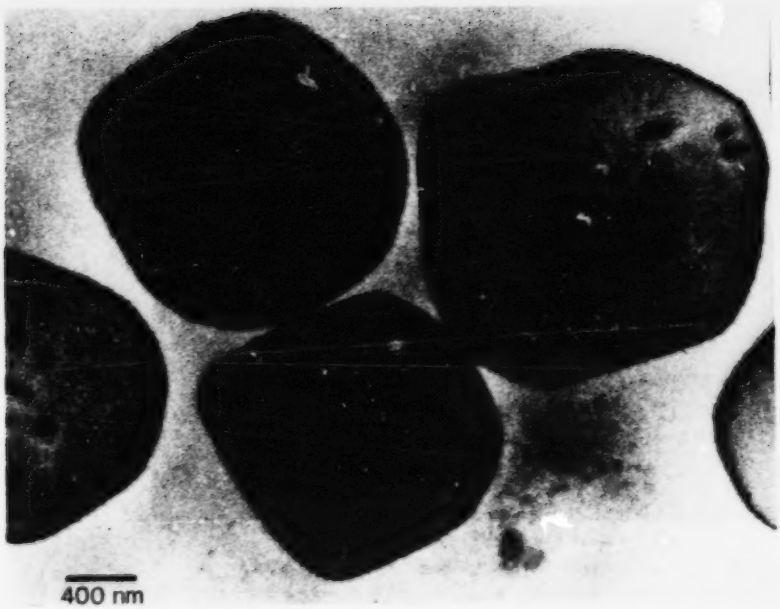

Figure 8. Inclusion bodies of a nuclear polyhedrosis virus of spruce budworm showing multicapsid virus particles, as seen under the electron microscope.

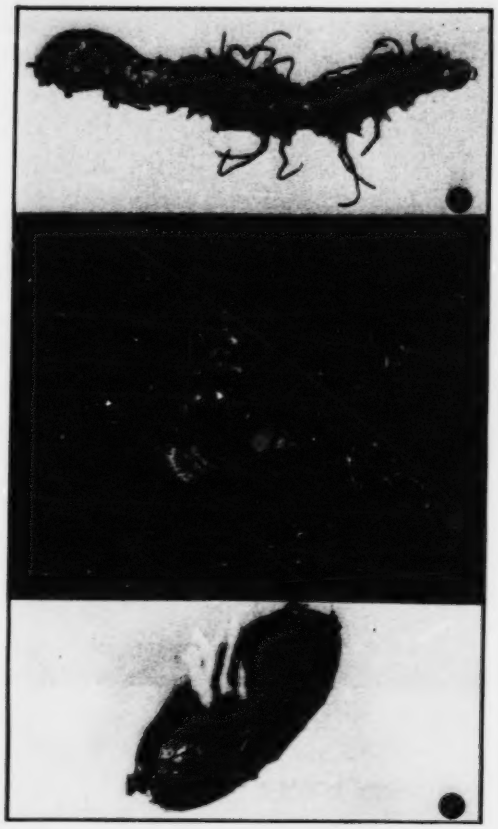

Figure 9. A) A spruce budworm larva, naturally infected with the fungus **Hirsutella gigantea**, showing fruiting bodies of varying length.

B) Actively discharged spores forming a halo around an adult flesh fly naturally infected with the fungus **Erynia bullata**.

C) The fungus **Paecilomyces farinosus** emerging from the cocoon of a larch sawfly.

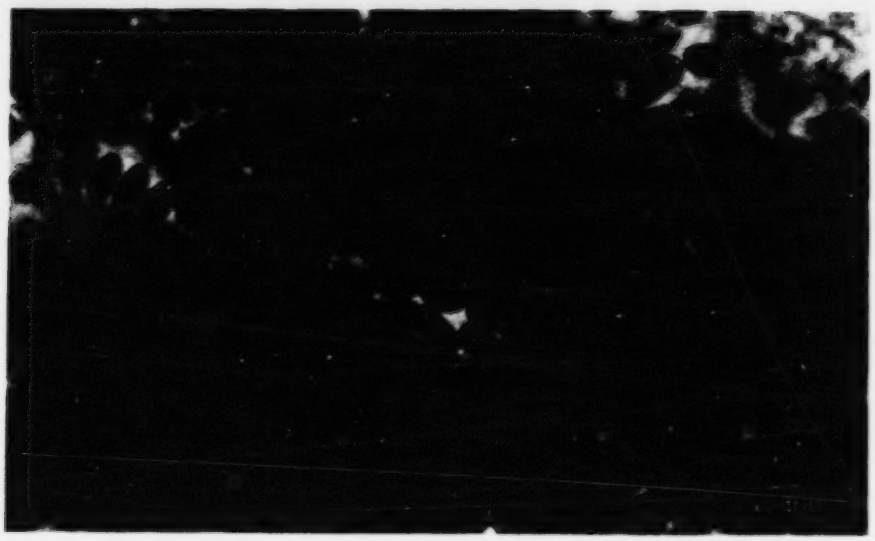

Figure 10. Spores of the microsporidium **Nosema fumiferanae** in gut tissue of the spruce budworm.